First description of germline mosaicism in familial hypertrophic cardiomyopathy

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Abstract

Familial hypertrophic cardiomyopathy is a genetically and phenotypically heterogeneous disease caused by mutations in seven sarcomeric protein genes. It is known to be transmitted as an autosomal dominant trait with rare de novo mutations.

A French family in which two members are affected by hypertrophic cardiomyopathy was clinically screened with electrocardiography and echocardiography. Genetic analyses were performed on leucocyte DNA by haplotype analysis with microsatellite markers at the *MYH7* locus and mutation screening by single strand conformation polymorphism analysis. Two subjects exhibited severe hypertrophic cardiomyopathy. A mutation in the *MYH7* gene was found in exon 14 (Arg453Cys). The two affected patients were carriers of the mutation, which was not found in the circulating lymphocytes of their parents. Haplotype analysis at the *MYH7* locus with two intragenic microsatellite markers (MYOI and MYOII) and the absence of the mutation in the father's sperm DNA suggested that the mutation had been inherited from the mother. However, it was not found in either her fibroblasts or hair.

This is the first description of germline mosaicism shown by molecular genetic analysis in an autosomal dominant disorder and more especially in hypertrophic cardiomyopathy. This mosaicism had been inherited from the mother but did not affect her somatic cells. Such a phenomenon might account for some de novo mutations in familial hypertrophic cardiomyopathy.

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Familial hypertrophic cardiomyopathy (FHC) is characterised by hypertrophy of the non-dilated left ventricle with predominant involvement of the interventricular septum (IVS) in the absence of other causes of hypertrophy.[1] It is a genetically and phenotypically heterogeneous disease transmitted as an autosomal dominant trait. There is considerable clinical heterogeneity and most patients are asymptomatic or have mild symptoms such as dyspnoea, chest pain, or lipothymia.[2] The major complications are sudden cardiac death[3] and severe heart failure. Clinical diagnosis of FHC is routinely based on ECG and echocardiographic abnormalities as described in children[4] and adults.[5] The genetic heterogeneity of this disease is illustrated by the identification of mutations in seven genes coding for sarcomeric proteins. Three contractile proteins are encoded by the β myosin heavy chain gene (*MYH7)* located on chromosome 14, the ventricular myosin essential light chain 1 gene (*MYL3)* on chromosome 3, and the ventricular myosin regulatory light chain 2 gene (*MYL2)* on chromosome 12. Three associated proteins are encoded by the cardiac troponin T gene (*TNNT2)* on chromosome 1, the cardiac troponin I gene (*TNNI3)* on chromosome 19, and the α tropomyosin gene (*TPM1)* on chromosome 15. The last protein is the cardiac myosin binding protein C encoded by the *MYBPC3* gene on chromosome 11.[6] All these proteins are involved in sarcomeric function and hypertrophy is considered to be a compensatory phenomenon to quantitative or qualitative abnormalities of the contractile process. We describe here the first case of germline mosaicism in a French family affected by FHC.

Methods

SUBJECTS

After informed consent was obtained under the guidelines of the Comité d'éthique du Centre Hospitalier Universitaire de la Pitié-Salpêtrière (Paris), all genotyped subjects underwent clinical and cardiovascular examination including a 12 lead electrocardiogram (ECG) and M mode, two dimensional, and Doppler echocardiography at the time of genotyping. The diagnosis of FHC was based on ECG or echocardiography or both.[5] The major echo diagnostic criterion was a 2D echo left ventricular end diastolic maximal wall thickness >13 mm. Major ECG abnormalities were a left ventricular hypertrophy (LVH) assessed by the Romhilt-Estes score, deep Q waves >0.04 sec or >1/3R wave, or significant ST-T changes. Two dimensional end diastolic left ventricular wall thickness measurements were obtained at different locations (anterior and posterior septum, anterolateral and posterior wall) from the parasternal short axis view at both the mitral valve and the papillary muscle levels and at the parasternal long axis view. The degree and extent of left ventricular hypertrophy were assessed by the maximal wall thickness and a scoring system proposed by Spirito and

Table 1 Clinical, ECG, and echocardiographic characteristics of the family. (Max IVS: maximal end diastolic interventricular wall thickness)

No	*Age (y)*	*Sex (M/F)*	*Symptoms*	*ECG abnormalities*	*Max IVS (mm)*
1	42	M	0	0	12
2	42	F	0	0	8
3	21	F	Dyspnoea (NYHA III)	Minor: deep S waves in leads V5V6	31
4	18	M	0	0	9
5	14	M	Dyspnoea (NYHA II) lipothymia	Minor: negative T wave (aVL)SV1+RV5 = 33 mm	25

Maron.[7] The distribution of hypertrophy was assessed according to the classification of Maron *et al*.[8]

GENETIC ANALYSIS

Genetic studies were first performed on genomic DNA extracted from circulating lymphocytes. Then, the DNA from the father's spermatozoa and the mother's fibroblasts and hair was investigated. The screening of mutations in the *MYH7* gene was performed by DNA single strand conformation polymorphism analysis (SSCP) after PCR amplification of each exon with primers designed in intronic flanking regions according to the sequence previously described.[9] Then the fragments with an abnormal profile were sequenced on both strands by cycle sequencing on an automated laser fluorescent DNA sequencer. In order to determine the parental allele carrying the mutation, haplotype analysis with microsatellite typing was performed with the two MYOI and MYOII intragenic *MYH7* markers.[10] Paternity testing with 17 polymorphic informative microsatellite markers located on chromosomes 11, 1, 15, and 12 was performed using standard techniques.

Results

Family Q is a French family with no history of sudden death. The nuclear family pedigree is shown in fig 1. Two subjects, Nos 3 and 5, were clinically affected and exhibited a typical and severe form of FHC. The clinical and echocardiographic characteristics of the two affected subjects, their brother, and parents are shown in table 1.

Subject 3 is a 21 year old woman. She underwent a pacemaker implantation in 1994 and a myotomy-myectomy in 1995 as she exhibited a symptomatic obstructive form of FHC despite medical treatment; however, she remains symptomatic. The ECG exhibits minor abnormalities but the interventricular septum remains dramatically hypertrophied (31 mm). Subject 5 is her 14 year old affected brother. He remains symptomatic despite medical treatment (a beta blocker agent). His ECG shows minor abnormalities and the maximal 2D echo left ventricular thickness is 25 mm. Subject 4 is the second brother. His clinical examination, ECG, and echocardiograph are normal and he is considered clinically unaffected. Subjects 1 and 2 are their parents. They are both asymptomatic and their ECG and echocardiographs are normal. Thus, they were considered to be clinically unaffected. The other 18 members were systematically screened. All showed normal clinical, ECG, and echocardiographic findings.

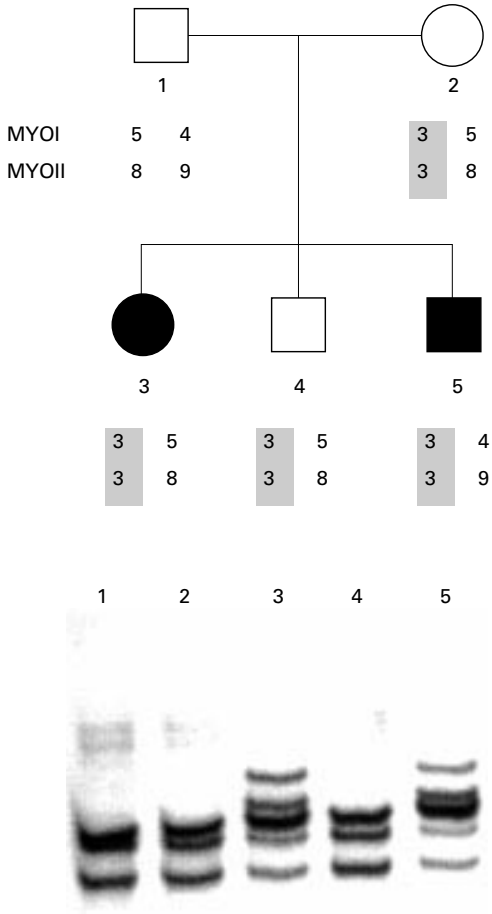

Figure 1 Family pedigree with haplotype and SSCP results. The two MYH7 gene intragenic markers MYOI and MYOII are listed from top to bottom. Inheritance of the 3-3 haplotype is shaded grey. The two affected children had the same maternal haplotype as did the unaffected child, which is compatible with germline mosaicism for a mutation in the MYH7 gene. Below the pedigree is the SSCP pattern of amplified exon 14. Lanes 1, 2, and 4 contain DNA fragments from the parents and unaffected child, lanes 3 and 5 contain DNA from the two affected children showing a nucleotide sequence change.

The SSCP screening of the first 24 *MYH7* exons followed by sequencing of the abnormal profiles enabled us to identify a C to T mutation in exon 14 leading to an Arg453Cys mutation in the protein. This Arg453Cys missense mutation was found only in subjects 3 and 5 but not in the brother, subject 4, or the two parents (fig 1). Paternity testing was done by haplotyping five loci with 17 polymorphic informative microsatellite markers and confirmed the compatibility. To determine if the mutation was transmitted by the father or the mother, we performed a haplotype analysis on chromosome 14 containing the *MYH7* locus and found that only the particular 3-3 haplotype transmitted by the mother was present in the two affected children. We checked that the father was not the mutation transmitter by testing the absence of the mutation in his sperm cells. Moreover, the mutation was absent in the mother's DNA from her somatic cells (fibroblasts, hair).

Discussion

We describe here a mutation in the *MYH7* gene, which seems to be associated with a severe form of early onset hypertrophic cardiomyopathy. Amino acid alignment across species and isoforms showed that this mutated residue located in the actin binding domain of the β myosin heavy chain is highly conserved, suggesting an important role for the function of the protein. This mutation had previously been described to be associated with a severe form of FHC.[11] Moreover, germline mosaicism is still unknown in cardiac diseases,[12] but was suspected in this family in which two children affected with a dominant disorder were born to phenotypically and genetically unaffected parents with no family history of hypertrophic cardiomyopathy. This hypothesis was confirmed because the Arg453Cys mutation was found in the two affected children but not in the parents. This germ cell mosaicism is of maternal origin and two possibilities should be considered. Either the mutation occurred through the mother in a germ cell that continued dividing or it occurred earlier in a cell before its separation into germinal cells and is therefore present in the mother's germinal and somatic cells.[13] In autosomal dominant diseases the probability of this second hypothesis is evaluated to be about 50%. To assess this hypothesis, we looked for the mutation in the mother's fibroblast and hair DNA. The degree of somatic mosaicism may vary according to the tissue and the mutation may be absent in white blood cells and detectable only in fibroblasts or other tissues, such as hair, muscle, and buccal smear.[14] Finally, we did not find the mutation in the mother's somatic cells and thus we describe here the first case of female germline mosaicism which is not associated with somatic mosaicism.

Female germline mosaicism has been reported in several X linked diseases, particularly in Duchenne muscular dystrophy (DMD),[15] but rarely in autosomal dominant disorders. Moreover, the few reports of female germline mosaicism in autosomal dominant diseases were all associated with somatic mosaicism. The only report of a potential female germline mosaicism with no somatic one was recently described in tuberous sclerosis[16] on the basis of the absence of the mutation in the mother's circulating lymphocytes. Thus, this report is the first description of a case of female germline mosaicism with no somatic mosaicism assessed by molecular genetic analysis in familial hypertrophy cardiomyopathy.

As this is a new phenomenon in cardiology, its extent cannot yet be evaluated but it might account for some apparently de novo mutations in families affected by the disease.[17] Up to now, genetic screening consisted of the search for mutations in lymphocyte DNA but has not been attempted in fibroblast DNA.[18] The published frequency of germline mosaicism in the case of de novo mutations varies widely depending on the clinical expression of mosaicism for different genes and on the type of mutations. It ranges from 0% in Apert syndrome to 11-20% in haemophilia A and B, Duchenne muscular dystrophy, or facioscapulohumeral dystrophy,[12] and about two thirds of cases are sporadic in tuberous sclerosis.[19] In the case of FHC, a dominant disease with incomplete penetrance, the knowledge that germline mosaicism may exist excludes the possibility of indirect diagnosis by linkage analysis and indicates that mutation identification is essential.[20] Thus, further investigations are necessary to evaluate the clinical importance and frequency of germline mosaicism in FHC and its consequences in terms of genetic counselling.[13]

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